

CLAIMS

1. An enzyme having the following properties:
 - a) exhibiting endoglucanase activity; and
 - b) capable of completely removing fuzz from regenerated cellulose fabrics at a concentration of 1 mg of the protein/L or below.
2. The enzyme according to claim 1, wherein its activity of fuzz removal from regenerated cellulose fabrics at pH 8.5 is 50% or more of its fuzz removal activity at the optimum pH.
3. The enzyme according to claim 1, wherein the enzyme is derived from *Zygomycotina*.
4. The enzyme according to any one of claims 1 to 3, wherein the enzyme is derived from the genus *Rhizopus* and has an average molecular weight of approximately 40 kD as determined by SDS-PAGE.
5. The enzyme according to claim 4, wherein the enzyme has the following properties:
 - the optimum pH for its activity of fuzz removal from regenerated cellulose fabrics: approximately 5; and
 - the optimum temperature for its activity of fuzz removal from regenerated cellulose fabrics: approximately 55°C.
6. The enzyme according to any one of claims 1 to 3, wherein the enzyme is derived from the genus *Mucor* and has an average molecular weight of approximately 41 kD as determined by SDS-PAGE.

7. The enzyme according to claim 6, wherein the enzyme has the following properties:

- the optimum pH for its activity of fuzz removal from regenerated cellulose fabrics: approximately 5-6; and
- the optimum temperature for its activity of fuzz removal from regenerated cellulose fabrics: approximately 50°C.

8. The enzyme according to any one of claims 1 to 3, wherein the enzyme is derived from the genus *Phycomyces* and has an average molecular weight of approximately 45 kD as determined by SDS-PAGE.

9. The enzyme according to claim 8, wherein the enzyme has the following properties:

- the optimum pH for its activity of fuzz removal from regenerated cellulose fabrics: approximately 6; and
- the optimum temperature for its activity of fuzz removal from regenerated cellulose fabrics: approximately 50°C.

10. An enzyme comprising a cellulose binding domain consisting of the following amino acid sequence (I) and exhibiting endoglucanase activity:

Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Gln-Cys-Gly-Gly-Xaa-Xaa-Xaa-Xaa-Gly-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Xaa-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Asn-Xaa-Xaa-Tyr-Xaa-Gln-Cys-Xaa (I) (SEQ ID NO: 18)

wherein Xaa is independently any amino acid residue; Xaa's at positions 20, 21, 22, 23, 24, 30 and 31 may be independently absent; and one of Xaa at position 11 or 33 is Lys and the other is any amino acid residue except Lys.

11. An enzyme comprising a cellulose binding domain consisting of the following

amino acid sequence (II) and exhibiting endoglucanase activity:

Cys-Ser-Xaa-Xaa-Tyr-Xaa-Gln-Cys-Gly-Gly-Xaa-Xaa-Trp-Xaa-Gly-Pro-Thr-Cys-Cys-Xaa
-Xaa-Gly-Xaa-Thr-Cys-Xaa-Xaa-Xaa-Xaa-Xaa-Asn-Xaa-Xaa-Tyr-Ser-Gln-Cys-Xaa (II)

(SEQ ID NO: 19)

wherein Xaa is independently any amino acid residue; and Xaa's at positions 20, 21, 23, 29 and 30 may be independently absent.

12. The enzyme according to claim 11, wherein one of Xaa at position 11 or 32 is Lys and the other is any amino acid residue except Lys.

13. The enzyme according to claim 10 or 11, wherein the cellulose binding domain consists of the following amino acid sequence (III):

Cys-Ser-X1-X2-Tyr-X3-Gln-Cys-Gly-Gly-X4-X5-Trp-X6-Gly-Pro-Thr-Cys-Cys-X7-X8-Gly-X9-Thr-Cys-X10-X11-X12-X13-X14-Asn-X15-X16-Tyr-Ser-Gln-Cys-X17 (III) (SEQ

ID NO: 20)

wherein:

X1 is Lys, Ser or Gln;

X2 is Leu, Ala, Val or Gly;

X3 is Gly, Tyr or Ser;

X4 is Lys or Ile;

X5 is Asn, Asp, Gly or Met;

X6 is Asn, Asp, Ser or Thr;

X7 is Glu, Asp or Thr;

X8 is Ser or Ala;

X9 is Ser or Phe;

X10 is Lys or Val;

X11 is Val, Asp, Ala or Gly;

X12 is Ser, Tyr, Gln or Ala;

X13 is Pro, Glu or Lys, or is absent;

X14 is Asp, Gly or Asn, or is absent;

X15 is Asp, Pro, Lys or Glu;

X16 is Tyr, Phe or Trp;

X17 is Leu, Val or Ile; and

one of X4 or X15 is Lys and the other is any amino acid residue except Lys.

14. The enzyme according to claim 10 or 11, wherein the cellulose binding domain consists of the following amino acid sequence (IV):

Cys-Ser-Lys-X21-Tyr-X22-Gln-Cys-Gly-Gly-Lys-X23-Trp-X24-Gly-Pro-Thr-Cys-Cys-Glu
-Ser-Gly-Ser-Thr-Cys-X25-X26-X27-X28-X29-Asn-X30-X31-Tyr-Ser-Gln-Cys-X32

(IV) (SEQ ID NO: 21)

wherein:

X21 is Leu or Ala;

X22 is Gly or Tyr;

X23 is Asn or Asp;

X24 is Asn or Asp;

X25 is Lys or Val;

X26 is Val or Asp;

X27 is Ser or Tyr;

X28 is Pro, or is absent;

X29 is Asp, or is absent;

X30 is Asp or Pro;

X31 is Tyr or Phe; and

X32 is Leu or Val.

15. The enzyme according to claim 14, wherein the cellulose binding domain consists of any one of the amino acid sequences of SEQ ID NOS: 22, 23, and 24.

16. The enzyme according to claim 10 or 11, wherein the cellulose binding domain consists of the following amino acid sequence (V):

Cys-Ser-Ser-Val-Tyr-X41-Gln-Cys-Gly-Gly-Ile-Gly-Trp-X42-Gly-Pro-Thr-Cys-Cys-X43-X

44-Gly-Ser-Thr-Cys-X45-Ala-Gln-X46-X47-Asn-Lys-Tyr-Tyr-Ser-Gln-Cys-X48 (V)
(SEQ ID NO: 25)

wherein:

Sub A5
X41 is Gly or Ser;
X42 is Ser or Thr;
X43 is Glu or Asp;
X44 is Ser or Ala;
X45 is Val or Lys;
X46 is Glu or Lys;
X47 is Gly or Asp; and
X48 is Leu or Ile.

17. The enzyme according to claim 16, wherein the cellulose binding domain consists of the amino acid sequence of SEQ ID NO: 26 or 27.

18. The enzyme according to claim 10 or 11, wherein the cellulose binding domain consists of the amino acid sequence of SEQ ID NO: 28.

19. The enzyme according to claim 10 or 11, wherein the cellulose binding domain is located on its N-terminal side.

Sub A6
20. The enzyme according to any one of claims 10 to 19, wherein the enzyme is capable of removing fuzz completely from regenerated cellulose fabrics at a concentration of 1 mg of the protein/L or below.

21. The enzyme according to any one of claims 10 to 20, wherein its activity of fuzz removal from regenerated cellulose fabrics at pH 8.5 is 50% or more of its fuzz removal activity at the optimum pH.

22. The enzyme according to any one of claims 10 to 21, wherein the enzyme is

derived from *Zygomycotina*.

Sub A6
23. The enzyme according to any one of claims 10 to 22, wherein the enzyme further comprises a part of its linker region consisting of the following amino acid sequence (VI):
Tyr-Xaa-Xaa-Xaa-X51-Gly-Gly-Xaa-X52-Gly (VI) (SEQ ID NO: 31)
wherein Xaa is independently any amino acid residue; and X51 and X52 are independently Ser or Thr.

24. The enzyme according to claim 23, wherein the part of the linker region consists of the following amino acid sequence (VII):

Tyr-X61-Xaa-X62-X51-Gly-Gly-Xaa-X52-Gly (VII) (SEQ ID NO: 32)

wherein:

Xaa is any amino acid residue;

X51 and X52 are independently Ser or Thr;

X61 is Lys or Ser; and

X62 is Ile or Val.

25. The enzyme according to claim 24, wherein Xaa at position 3 is Ala, Ile, Pro or Val, and Xaa at position 8 is Ala, Phe or Lys.

Sub A7
26. The enzyme according to any one of claims 23 to 25, wherein both X51 and X52 are Ser.

27. The enzyme according to claim 23, wherein the part of the linker region consists of any one of the sequences as shown in SEQ ID NOS: 33, 34, 35, 36 and 37.

Sub A8
28. The enzyme according to any one of claims 23 to 27, wherein the part of the linker region is located on the N-terminal side of the catalytic domain.

29. An enzyme which comprises a part of its linker region consisting of the

following amino acid sequence (VI) and exhibits endoglucanase activity:

Tyr-Xaa-Xaa-Xaa-X51-Gly-Gly-Xaa-X52-Gly (VI) (SEQ ID NO: 31)

wherein Xaa is independently any amino acid residue; and X51 and X52 are independently Ser or Thr.

30. The enzyme according to claim 29, wherein the part of the linker region consists of the following amino acid sequence (VII):

Tyr-X61-Xaa-X62-X51-Gly-Gly-Xaa-X52-Gly (VII) (SEQ ID NO: 32)

wherein:

Xaa is any amino acid residue;

X51 and X52 are independently Ser or Thr;

X61 is Lys or Ser; and

X62 is Ile or Val.

31. The enzyme according to claim 30, wherein Xaa at position 3 is Ala, Ile, Pro or Val and Xaa at position 8 is Ala, Phe or Lys.

Sub A9 ~~32. The enzyme according to any one of claims 29 to 31, wherein both X51 and X52 are Ser.~~

33. The enzyme according to claim 29, wherein the part of the linker region consists of any one of the sequences as shown in SEQ ID NOS: 33, 34, 35, 36 and 37.

Sub A10 ~~34. The enzyme according to any one of claims 29 to 33, wherein the enzyme is capable of removing fuzz completely from regenerated cellulose fabrics at a concentration of 1 mg of the protein/L or below.~~

35. The enzyme according to any one of claims 29 to 34, wherein its activity of fuzz removal from regenerated cellulose fabrics at pH 8.5 is 50% or more of its fuzz removal activity at the optimum pH.

Sub A10
 36. The enzyme according to any one of claims 29 to 35, wherein the enzyme is derived from *Zygomycotina*.

37. An endoglucanase having the following characteristics:
 i) belonging to family 45;
 ii) being derived from a filamentous fungus; and
 iii) having a cellulose-binding domain located on its N-terminal side.

Sub A11
 38. The enzyme according to claim 37, wherein the cellulose-binding domain consists of the amino acid sequence described in any one of claims 10 to 18.

39. The enzyme according to claim 37, wherein the filamentous fungus belongs to the genus *Rhizopus*, the genus *Mucor* or the genus *Phycomyces*.

40. A protein comprising the amino acid sequence as shown in SEQ ID NO: 1, 3, 5, 7, 9 or 11; a modified protein thereof exhibiting endoglucanase activity; or a homologue of said protein or said modified protein.

Sub A12
 41. The enzyme according to any one of claims 1 to 39 or the modified protein according to claim 40, wherein the enzyme or the modified protein is modified so that Asn-linked oligosaccharide chains are not added thereto.

42. The modified protein according to claim 41, wherein the modification is the replacement of Asn, Ser and/or Thr with other amino acid(s) and/or the replacement of Xaa with Pro in its Asn-linked glycosylation site(s) Asn-Xaa-Ser/Thr where Xaa is any amino acid residue.

43. The modified protein according to claim 41, wherein the modification is the replacement of Asn with Asp or Gln, and/or the replacement of Ser or Thr with Ala, Gly or

Leu, and/or the replacement of Xaa with Pro in its Asn-linked glycosylation site(s) Asn-Xaa-Ser/Thr where Xaa is any amino acid residue.

44. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1 where the amino acid residue at position 45 or 47 is replaced by other amino acid residue.

45. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1 where the amino acid residue at position 45 or 47 and the amino acid residue(s) at position 90 or 92 and/or position 130 or 132 are replaced by other amino acid residues.

46. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 3 where the amino acid residue at position 45 or 47 is replaced by other amino acid residue.

47. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 3 where the amino acid residue at position 45 or 47 and the amino acid residue(s) at position 92 or 94, position 119 or 121, position 122 or 124 and/or position 158 or 160 are replaced by other amino acid residues.

48. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 5 where the amino acid residue at position 44 or 46 is replaced by other amino acid residue.

49. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 5 where the amino acid residue at position 44 or 46 and the amino acid residue(s) at position 49 or 51, position 121 or 123 and/or position 171 or 173 are replaced by other amino acid residues.

50. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 7 where the amino acid residue at position 50 or 52 is replaced by other amino acid residue.

51. The modified protein according to claim 41, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 9 where the amino acid residue at position 99 or 101 is replaced by other amino acid residue.

52. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 where the cellulose binding domain is modified.

53. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 where a part of the linker domain is modified.

54. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 where the cellulose binding domain and a part of the linker region are modified.

Sub A3
~~55. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 which is modified so that the cellulose binding domain represents one of the amino acid sequences (I) to (V) described in claims 10, 11, 13, 14 and 16, respectively, and which may have a modification(s) in a region(s) other than said cellulose binding domain.~~

56. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 which is modified so that a part of the linker region represents the amino acid sequence (VI)

Sub A13 or (VII) described in claim 23 or 24 and which may have a modification(s) in a region(s) other than said part of the linker region.

~~57. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 which is modified so that the cellulose binding domain represents one of the amino acid sequences (I) to (V) described in claims 10, 11, 13, 14 and 16, respectively, and yet modified so that a part of the linker region represents the amino acid sequence (VI) or (VII) described in claim 23 or 24, and which may have a modification(s) in a region(s) other than said cellulose binding domain and said part of the linker region.~~

58. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 which is modified in a region(s) other than the cellulose binding domain and a part of the linker region.

59. The modified protein according to claim 40, wherein said modified protein consists of a modified amino acid sequence represented by SEQ ID NO: 1, 3, 5, 7, 9 or 11 which is modified in a region(s) other than the cellulose binding domain, a part of the linker region and the catalytic domain.

Sub A14 ~~60. A polynucleotide comprising a nucleotide sequence encoding the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59.~~

61. The polynucleotide according to claim 60, wherein said polynucleotide comprises the DNA sequence as shown in SEQ ID NO: 2, 4, 6, 8, 10 or 12, or a modified sequence thereof.

Sub A15 ~~62. The polynucleotide according to claim 60 or 61, wherein said polynucleotide comprises a nucleotide sequence in which codons have been optimized for a host by~~

Sub A15 selecting those ~~/~~ codons frequently used by the host.

63. The polynucleotide according to claim 62, wherein said nucleotide sequence in which codons have been optimized is the DNA sequence as shown in SEQ ID NO: 13.

Sub A16 ~~64.~~ An expression vector comprising the polynucleotide according to any one of claims 60 to 63.

65. A host cell transformed with the polynucleotide according to any one of claims 60 to 63 or the expression vector according to claim 64.

66. The host cell according to claim 65, wherein said host cell is a yeast or filamentous fungus.

67. The host cell according to claim 66, wherein the yeast belongs to the genus *Saccharomyces*, the genus *Hansenula* or the genus *Pichia*.

68. The host cell according to claim 66, wherein the yeast is *Saccharomyces cerevisiae*.

69. The host cell according to claim 66, wherein the filamentous fungus belongs to the genus *Humicola*, the genus *Aspergillus*, the genus *Trichoderma*, the genus *Acremonium* or the genus *Fusarium*.

70. The host cell according to claim 66, wherein the filamentous fungus is *Humicola insolens*, *Aspergillus niger* or *Trichoderma viride*.

Sub A17 ~~71.~~ A method for producing the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59, comprising cultivating the host cell according to any one of claims 65 to 70 and recovering the enzyme, protein, modified protein or homologue

from said host cell and/or the resultant cultivation broth.

72. An endoglucanase produced by the method according to claim 71.

73. A cellulase preparation comprising the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72.

74. A method of treating cellulose-containing fabrics, comprising a step of contacting the cellulose-containing fabrics with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

75. A method of reducing the rate at which cellulose-containing fabrics become fuzzy or for reducing fuzzing in cellulose-containing fabrics, comprising a step of contacting the cellulose-containing fabrics with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

76. A method of weight loss treatment for cellulose-containing fabrics to improve its touch and appearance, comprising a step of contacting the cellulose-containing fabrics with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

77. A method of providing color clarification of colored cellulose-containing fabrics, comprising a step of treating the colored cellulose-containing fabrics with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

78. A method of providing a localized variation in color of colored cellulose-containing fabrics, comprising a step of treating the colored cellulose-containing

fabrics with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

79. A method of reducing the rate at which cellulose-containing fabrics become stiff or reducing stiffness in cellulose-containing fabrics, comprising a step of treating the cellulose-containing fabrics with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

80. The method according to any one of the claims 74 to 79, wherein the treatment of the fabrics is performed through soaking, washing or rinsing the fabrics.

81. An additive to detergent comprising the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73 in a non-scattering granular form or a stabilized liquid form.

82. A detergent composition comprising the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

83. A method of improving the freeness of a paper pulp, comprising a step of treating the paper pulp with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.

84. A method of deinking a waste paper, comprising a step of treating the waste paper with the endoglucanase, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73 in the presence of a deinking agent.

85. A method of improving the digestibility of an animal feed, comprising a step of

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treating a cellulose-containing feed with the enzyme, protein, modified protein or homologue according to any one of claims 1 to 59 and 72 or the cellulase preparation according to claim 73.